ผลของความหนาแน่นและพื้นที่แผ่นหลบซ่อนที่มีต่ออัตราการเจริญเติบโตและอัตราการรอดของหอยเป๋าฮื้อชนิด *Haliotis asinina*

Effect of stocking density and shelter surface area on growth and survival of the tropical abalone (*Haliotis asinina*) in a semi-flow through system

Tippawan Tantawanich[1], Wenresti G. Gallardo[1], Kou Ikejima[1],
Monthon Ganmanee[2] and Padermsak Jarayabhand[3]
[1]Aquaculture and Aquatic Resources Management, School of Environment and Resource Development, Asian Institute of Technology, P.O. Box 4, Klong Luang, Pathumthani 12120
[2] Department of Fisheries Science, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Chalongkrung Rd., Ladkrabang, Bangkok 10520
[3] Aquatic Resources Research Institute, Chulalongkorn University, Phaya thai Rd., Bangkok 10330

บทคัดย่อ

หอยเป๋าฮื้อไทยชนิด *Haliotis asinina* เป็นหอยทะเลเขตร้อน ซึ่งถือว่ามีศักยภาพสูงที่จะนำมาเป็นสัตว์น้ำเศรษฐกิจตัวใหม่ ซึ่งจำเป็นต้องมีการศึกษาวิจัยด้านเทคนิคการเลี้ยงเพื่อพัฒนาไปสู่การเลี้ยงในเชิงพาณิชย์อย่างสมบูรณ์ การทดลองนี้เป็นการศึกษาผลของความหนาแน่นและพื้นที่แผ่นหลบซ่อนที่มีต่ออัตราการเจริญเติบโตและอัตรารอดของหอยเป๋าฮื้อที่เลี้ยงในระบบการทำฟาร์มบนบกในระบบน้ำหมุนเวียนแบบกึ่งปิด โดยใช้หอยเป๋าฮื้อที่มีน้ำหนักเฉลี่ยเริ่มต้น 1.42 กรัม และมีความยาวเปลือกเฉลี่ย 18.7 มิลลิเมตร เลี้ยงที่ความหนาแน่น 100, 200 และ 300 ตัว/ตร.ม. และใส่แผ่นหลบซ่อนรูปตัว “V” จำนวน 1, 2 และ 3 แผ่น (0.09, 0.18 และ 0.27 ตร.ม.) โดยมีระยะเวลาการเลี้ยง 201 วัน

ผลการศึกษาพบว่า ในช่วง 5 เดือนแรกของการเลี้ยง อัตราการเจริญเติบโตจำเพาะในด้านน้ำหนัก, ความยาวและความกว้างเปลือกไม่มีความแตกต่างกันทางสถิติในทุกชุดการทดลอง ในช่วงท้ายของการทดลอง อัตราการเจริญเติบโตจำเพาะในด้านน้ำหนัก ความยาวและความกว้างเปลือกไม่มีความแตกต่างกันทางสถิติระหว่างชุดการทดลองที่มีแผ่นหลบซ่อนทั้ง 3 แบบ ที่เลี้ยงด้วยความหนาแน่น 100 และ 200 ตัว/ตร.ม. แต่ในชุดการทดลองที่เลี้ยงด้วยความหนาแน่น 300 ตัว/ตร.ม. และใส่แผ่นหลบซ่อน 3 แผ่น มีอัตราการเจริญเติบโตจำเพาะในด้านน้ำหนักและความยาวเปลือกสูงที่สุด เช่นเดียวกับชุดการทดลองที่เลี้ยงด้วยความหนาแน่น 300 ตัว/ตร.ม.และใส่แผ่นหลบซ่อน 2 แผ่น มีอัตราการเจริญเติบโตจำเพาะในด้านความยาวเปลือกแตกต่างกับชุดที่เลี้ยงที่ใส่แผ่นหลบซ่อน 1 แผ่นอย่างมีนัยสำคัญทางสถิติ ส่วนอัตราการเจริญเติบโตจำเพาะในด้านความกว้างเปลือกไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติในทุกชุดการทดลอง จากผลการทดลองชี้ให้เห็นว่าการเลี้ยงด้วยความหนาแน่น 300 ตัว/ตร.ม. จะมีความต้องการใส่แผ่นหลบซ่อนจำนวน 3 แผ่น ตั้งแต่เดือนที่ 6 เป็นต้นไป

Abstract

The tropical abalone *Haliotis asinina*, is a highly valued marine mollusk with very high export potential but its culture techniques still need to be developed. An experiment was conducted to investigate the effect of stocking density and shelter surface area on the growth and survival of abalone reared in fiberglass tank with a semi-flow through system. Abalone with average initial body weight of 1.42 g and shell length 18.7 mm were stocked at stocking densities of 100, 200 and 300 abalone m^{-2} with 1, 2 or 3 pieces of V-shaped PVC shelters added to the tanks for 201 days.

In the first 5 months of culture, there was no significant difference ($P>0.05$) in specific growth rates (SGR) in body weight (W), shell length (SL) and shell width (SW) among treatments. At the end of culture period, there was no significant difference in SGR among 3 shelter surface areas (0.09, 0.18 and 0.27 m^2) at stocking densities of 100 and 200 pcs. m^{-2}, but at 300 pcs. m^{-2} with 3 shelters, abalone had significantly higher SGR_W and SGR_{SL} ($P<0.05$). Also at 300 pcs. m^{-2} with 2 shelters, abalone had significantly higher SGR_{SL} ($P<0.05$). There was no significant difference in SGR_{SW} at different stocking densities and shelter surface areas throughout the culture period. These results indicate that at higher stocking density of 300 pcs. m^{-2}, at least 3 shelters are needed on the sixth month.

Introduction

Abalone are the most popularly maricultured gastropod in Southeast Asia. Live abalone have a high market demand and fetch high prices in Japan, Taiwan, Australia, New Zealand, the United States, Mexico and South Africa (Uki, 1989). Thailand is one of the principal countries that produce abalone for consumption and commerce (Jarayabhand and Paphavasit, 1996). The tropical abalone, *Haliotis asinina* is found on the islands along the eastern coasts of the upper Gulf of Thailand (Tookwinas et al., 1986). *H. asinina* has fast growth rates and a relatively high salinity tolerance in comparison to other species (Jarayabhand and Paphavasit, 1996). Statistics on total catches are not available, but there is a possibility that natural stocks will decline in the future due to overfishing and habitat destruction. Owing to rapid depletion of some stocks, many studies have been conducted in Thailand and other countries to develop effective artificial propagation and culture techniques.

Tropical abalone culture in Thailand is at the very early stages of basic and applied research conducted in small-scale operations (Jarayabhand and Paphavasit, 1996). The economic viability of commercial tropical abalone farming depends on the system design and techniques used for grow-out culture. Due to the long culture period and slow growth, profit can be marginal in land-based system if productivity of unit volume of tank. Hence, it is important to test alternative means of growing abalone juveniles to marketable size, such as culture tank design, number of shelters, and stocking density to minimize production costs. Abalone culture using shelters is practiced in many countries. The use of shelters can reduce the need to shade the tanks, increase the effective surface area of the tank, provide suitable culture condition that more closely replicates their natural environment, and may lead to better disease resistance (Hindrum et al., 1998). A higher surface area to volume ratio has been found to produce increased growth rates. The effective surface area within deeper flow through systems has been further enhanced by the placement of internal bricks or plastic structures (Benson et al, 2001).

The factors known to influence the growth of abalone juveniles are water flow, depth, water quality parameters, food quantity and quality, stocking density and the tank system. However, the effects of stocking density and shelter surface area have not received much attention despite the fact that grazing gastropods such as abalone are known to show density-dependent growth (Huchette et al., 2003).

The present study was conducted to investigate the effect of stocking density and shelter surface area on the growth and survival of abalone reared in fiberglass tank with a semi-flow through system.

Materials and Methods

1. Location

The study was conducted at the Sichang Marine Science Research and Training Station (SMART), Chulalongkorn University, Bangkok, Thailand.

2. Experimental animals

The *H. asinina* juveniles initially stocked in September 2005 were 5 months old, 1.42±0.56 g (mean ± SD) in weight and 1.87 ± 0.19 cm in length. They were produced by artificial spawning at SMART. One week before the start of the experiments, the abalone were acclimatized by stocking in the experimental tanks provided with shelters. A total of 2,700 juveniles were stocked in the 27 tanks. In October 2005, fifty abalone per treatment (total of 1,350 pcs.) were tagged individually by using Dymo tape and epoxy cement. Individual tagging was used to reduce sampling error and provide more accurate information than the usual technique of random sampling.

3. Experimental diets and feeding

During the experiment, the abalone were fed an artificial diet (25 % crude protein) produced by SMART using seaweed powder, soy bean powder and fish meal as main ingredients. The artificial diet pellets were broken into small pieces and uniformly distributed inside each shelter. Food was given once a day at 1800H at a rate of 2.0-2.5% of total body weight.

4. Shelter

Shelters made from PVC plate (30 x 30 cm, 1.5 mm thickness) formed into V-shape were used in this experiment. This experiment used three levels of total underside surface shelter area of 0.09, 0.18 and 0.27 m^2/tank, respectively.

5. System design

All abalone were reared in 250-L fiberglass tanks, each tank had 0.50 m^2 bottom surface area and filled with 200-L seawater and provided with roof-shaped shelters. They were stocked at a density of 100, 200, and 300 abalone m^{-2}. About 2,700 juveniles were fed with artificial diet throughout the experiment and reared in a semi-flow through system tank. Addition of clean seawater was at a rate of 2.54 liters/min. Aeration was provided throughout the experiment.

6. Experimental design

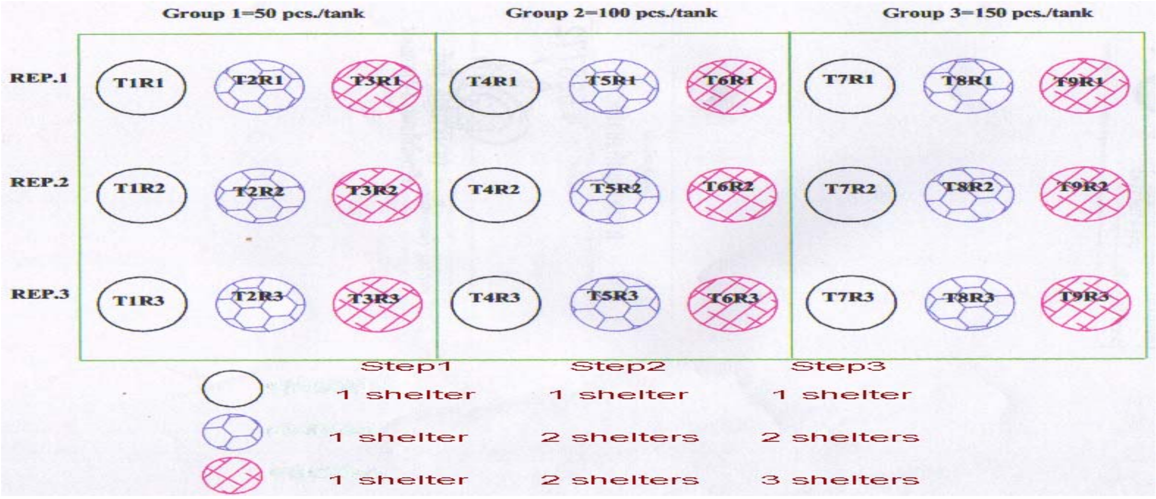

Figure1. Schematic diagram of the experimental design.

<u>Step 1</u>: The abalone in all groups were reared in tanks with one shelter from October-November 2005. (Fig. 1)

Group 1 (T1R1-T3R3): 100 pcs./m2; 9 replicates

Group 2 (T4R1-T6R3): 200 pcs./m2; 9 replicates

Group 3 (T7R1-T9R3): 300 pcs./m2; 9 replicates

Step 2: After the second month, one shelter was added in T2R1-T3R3, T5R1-T6R3 and T8R1-T9R3, and rearing was from December 2005 to January 2006.

T2R1-T3R3: 100 pcs./m2; two shelters/tank; 6 replicates
T5R1-T6R3: 200 pcs./m2; two shelters/tank; 6 replicates
T8R1-T9R3: 300 pcs./m2; two shelters/tank; 6 replicates

Step 3: After the fourth month, another shelter was added in T3R1-T3R3, T6R1-T6R3 and T9R1-T9R3, and rearing was from February to March 2006.

T3R1-T3R3:100 pcs./m2; three shelters/tank; 3 replicates
T6R1-T6R3:200 pcs./m2; three shelters/tank; 3 replicates
T9R1-T9R3:300 pcs./m2; three shelters/tank; 3 replicates

This experiment used 3x3 factorial in completely randomized design (CRD).

7. Abalone data collection and analysis

Growth in terms of body weight, shell length and width was determined every 2 months. An electronic balance was used to register the weight in grams, and a vernier caliper was used to measure the shell length and width to the nearest 1 mm. Dead abalone were removed and counted everyday and survival rates were calculated every month.

The formula used to calculate specific growth rate in weight, shell length and width (% per day) and survival rate (%) are as follows:

2.1 Specific growth rate in weight (SGR_W):

$$SGR_W = \{(\ln W_T - \ln W_t)/(T-t)\} \times 100$$

W_T is weight at time T; W_t is weight at time t; $(T-t)$ is time in days between weightings.

2.2 Specific growth rate in shell length (SGR_{SL}):

$$SGR_{SL} = \{b(\ln L_T - \ln L_t)/(T-t)\} \times 100$$

L_T was shell length at time T; L_t is shell length at time t;

b was the slope of weight-length relationship of the form, $W = aL^b$. The coefficient b was obtained by using a non-linear regression model which implements Marquardt’s algorithm (Saila et al., 1988).

2.3 Survival rate: $N_t / N_i \times 100$

N_t = total number of abalone at t days, N_i = initial total number of abalone.

8. Statistical analysis

Differences in growth and survival rates among the different stocking densities and shelter surface areas were analyzed by one-way ANOVA for each stocking density and shelter area as a factor, using SPSS (Version 13.0) statistical software package (SPSS Inc., Chicago, USA). Tukey's test was used to identify significant differences between treatments. SPSS and Excel program were used in regression analysis of the relationship between growth and stocking density and shelter surface area.

Results

1. Growth performance of abalone

Effect of stocking density

In the first 5 months of culture (September-January), there was no significant difference ($P>0.05$) in specific growth rates (SGR) for body weight (BW), shell length (SL) and shell width (SW) among treatments. However, comparing the SGR between stocking densities at each number of shelters on each month, SGR_{BW} between January and March was significantly affected by stocking density. Abalone had higher SGR_{BW} when 3 shelters were used at abalone density of 300 pcs./m^2 ($P<0.05$, Fig. 2) but no significant difference in SGR for SL, SW under any treatment on any month.

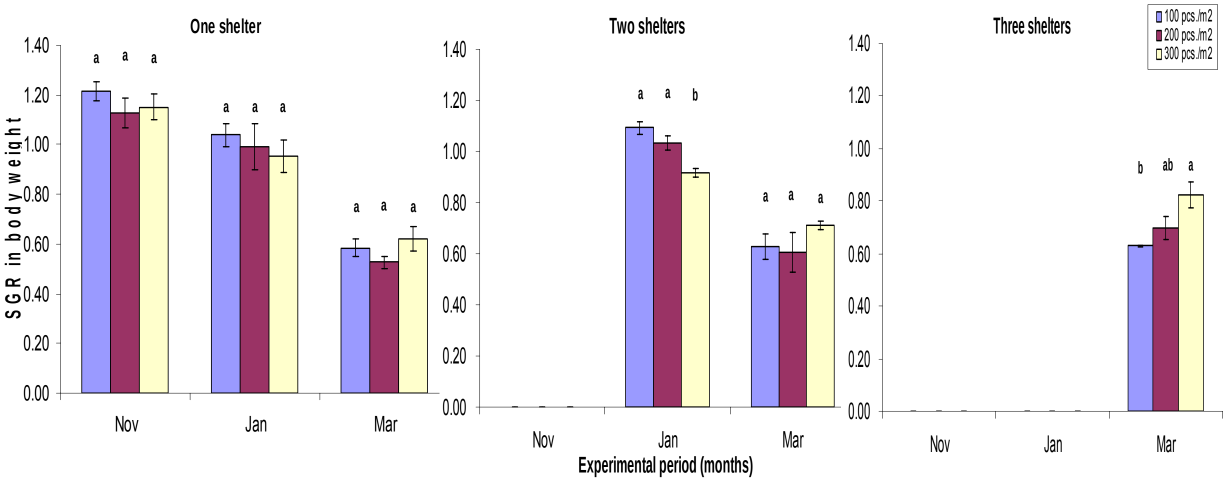

Figure 2. Specific growth rate in body weight of abalone cultured for 201 days at 100 pcs./m^2 (A), 200 pcs./m^2(B) and 300 pcs./m^2 (C) with 1, 2 and 3 shelters (0.09, 0.18 and 0.27 m^2), respectively. Bars with different superscripts in a month are significantly different at $P<0.05$.

Effect of shelter surface area

In the first 5 months of culture, there was no significant difference ($P>0.05$) in SGR (BW, SL, SW) among treatments but at the end of the rearing period (March) the abalone had significantly higher

(P<0.05) SGR_{BW} at 300 pcs./m^2 with three shelters (0.27 m^2) (Fig. 3), and SGR_{SL} at 300 pcs./m^2 with two and three shelters (0.18 m^2 and 0.27 m^2, respectively) (Fig. 4). There was no significant difference in SGR_{SW} at different shelter surface areas and different stocking densities throughout the culture period.

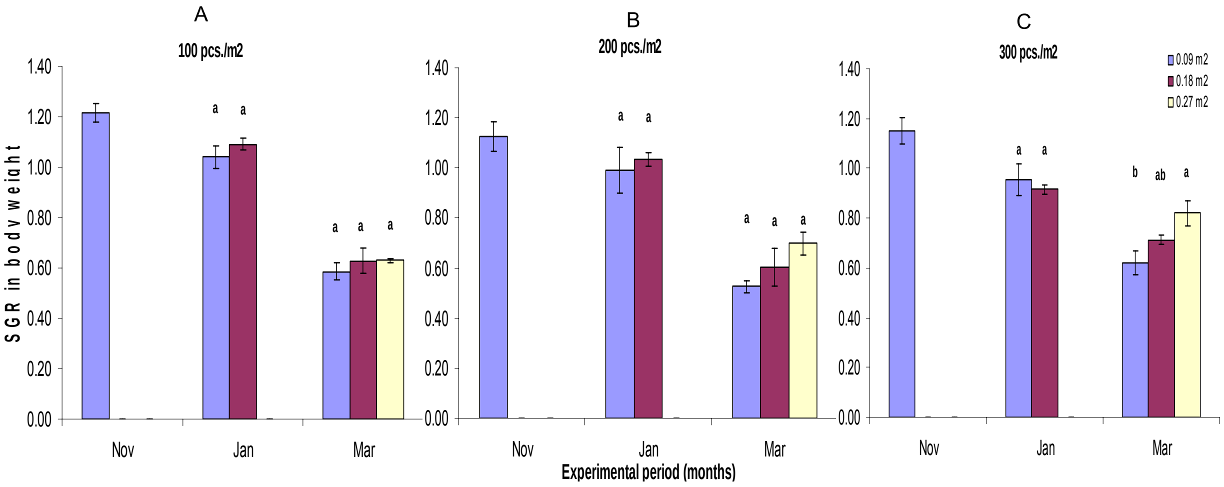

Figure 3. Specific growth rate in body weight of abalone cultured for 201 days at 100 pcs./m^2 (A), 200 pcs./m^2(B) and 300 pcs./m^2 (C) with 1, 2 and 3 shelters (0.09, 0.18 and 0.27 m^2), respectively. Bars with different superscripts in a month are significantly different at P<0.05.

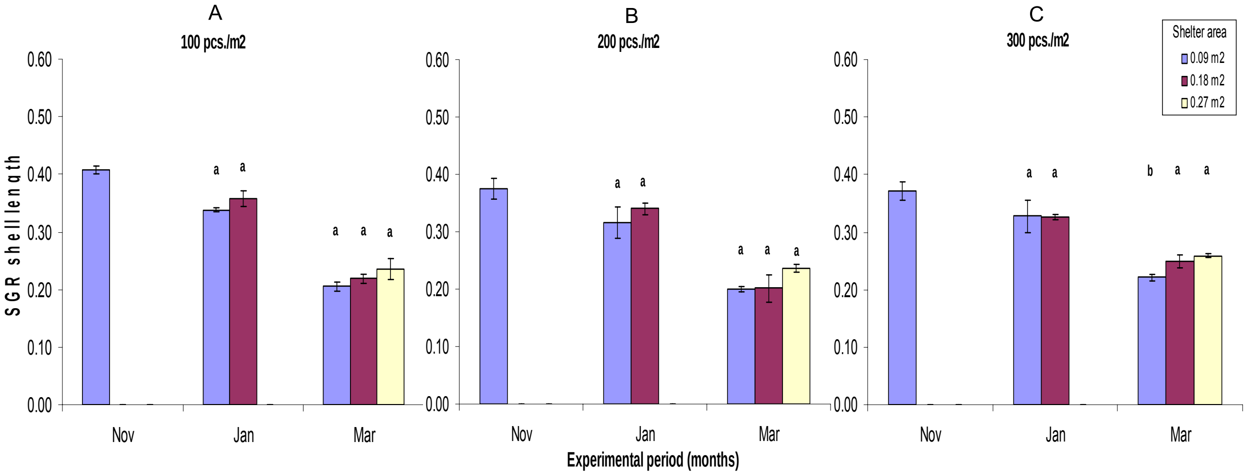

Figure 4. Specific growth rate in shell length of abalone cultured for 201 days at 100 pcs./m^2 (A), 200 pcs./m^2(B) and 300 pcs./m^2 (C) with 1, 2 and 3 shelters (0.09, 0.18 and 0.27 m^2), respectively. Bars with different superscripts in a month are significantly different at P<0.05.

2. Survival rate

On February-March abalone had swollen gonad and rotten foot caused by bacteria and this resulted in mortalities and low survival rates of 40-60% at the end of culture period. Tanks with stocking densities of 200 and 300 pcs./m^2 with 2 shelters had significantly lower survival (Fig. 5).

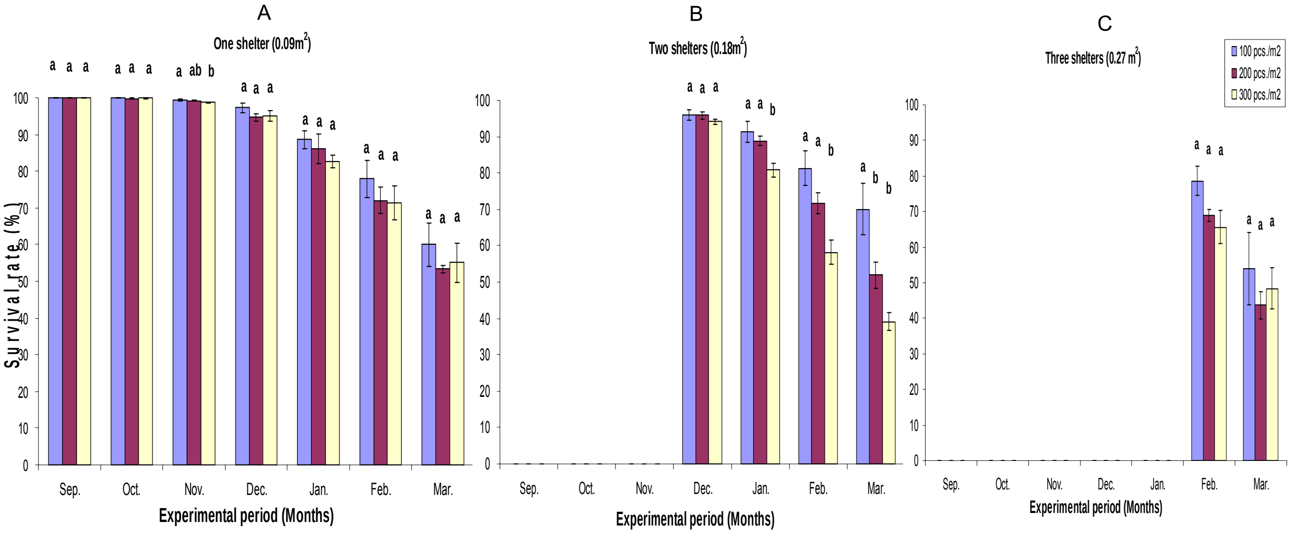

Figure 5. Survival rates of abalone cultured for 201 days with 1 shelter (0.09m^2) (A), 2 shelters (0.18 m^2) (B) and 3 shelters (0.27 m^2) (C) at 100, 200 and 300 pcs./m^2. Bars with different superscripts in a month are significantly different at P<0.05.

Discussions

1. Growth performance

The results showing no significant difference in SGR in the first 5 months indicate that only one shelter is enough in the first 5 months of culture even at high densities of 300 pcs/m^2, suggesting that abalone are still small and they are not overcrowded. The lower SGR_{BW} when 1 or 2 shelters are used at abalone density of 300 pcs./m^2 shows that more shelters are needed after 5 months of culture because abalone tend to stack especially at high densities due to lack of primary attachment space to move and ingest food, thus affecting their feeding rate even though there is enough food (Mgaya and Mercer, 1995).

Comparing the SGR_{BW} between stocking densities at 3 shelters, abalone had higher SGR at stocking density of 300 pcs./m^2 (Fig. 4.1). In February mass mortalities occurred due to bacterial disease particularly in tanks with 200 and 300 pcs./m^2 but feeding rate was not lowered thereafter because there was no remaining food in the tanks in the morning. This suggests that the food was not enough even at lower stocking densities, thus, growth was lower at stocking densities of 100 and 200 pcs./m^2, while growth increased significantly in the supposedly high density of 300 pcs./m^2 The feeding rates (2.0-2.5%BW) that were used in this experiment should be adjusted.

Since the difference in growth between treatments was significant only in the later part of the experimental period the overall relationship between stocking density and growth was not statistically significant but there was a trend of decreasing growth with increasing stocking density. The inverse relationship between growth and stocking density suggests that there is density-dependent competition for space or food (Jarayabhand and Newkirk, 1989).

The culture period of 7 months was not enough to produce cocktail size abalone (20-100 g or 3.8-7.6 cm shell length). This may due to the fact that the experiment started during the rainy season (September-November) and the salinity decreased to 27‰ which is below the optimum (30-35‰), and November to January was a winter season with lower temperature. So, abalone had more stress and declined in feeding rate during the supposedly faster-growing phase. Furthermore, the artificial diet used had high level of crude protein (25%) which may cause faster gonad development. Several studies have noted that the growth rate of abalone declines after sexual maturation (Shepherd and Hearn, 1983) and a direct relationship between food consumption and gonad development (Taylor et al., 1997). The increased food intake compensated for the energy that was used for gonad maturation and spawning.

2. Survival rate

Comparing the survival rates between stocking densities at each number of shelter (Fig. 4.4), abalone had lower survival at higher density of 200 and 300 pcs./m^2 with two shelters (0.18 m^2) but with three shelters, survival rates did not differ between stocking densities. It could be expected that the survival would be lower at higher densities of 200 and 300 pcs./m^2 when there is only one shelter but in this experiment, this is not so because with only one shelter, water circulation may be better, thus, less problem with bacterial disease in tanks with only one shelter. In February-March abalone had swollen gonad and rotten foot caused by bacteria and this resulted in mortalities and low survival rates. Antibiotic was not used in this experiment because it could actually be harmful to the environment.

In Tasmania, Australia, disease outbreaks among cultured abalone (*H. rubra* and *H. laevigata*) were associated with two species of *Vibrio* (*V. harveyi* and *V. splendidus* I). In most cases, stress factors (e.g., high temperatures, grading trauma, anesthetics, gradual increase in salinity in the recirculation system, etc.) were reported to have precipitated the diseases (Handlinger et al., 2002). Another important factor that may affect abalone survival is the rate of water flow. Since feeding was done daily, those at higher densities received higher loads of feed which restricted water movement within the fiberglass tank. Concentrations of total ammonia nitrogen tended to be higher from December onwards. Growth and survival rate of abalone is inhibited by increased levels of metabolic wastes and reduced dissolved oxygen (Hahn, 1989; Fallu, 1991); hence, high water exchange rate is important to maintain water quality as stocking densities increase (Aviles and Shepherd, 1996).

Further study should be done on the abalone diseases in order to increase survival rate because most of abalone in the experiment died cause from swollen gonad and stomach and rotten foot. Furthermore, it should be consider on the capital of culture at different stocking densities and shelter surface areas in appropriately season to produce cocktail size or steak size abalone.

Acknowledgements

The author is greatly indebted to the Royal Thai Government Fellowship and the Thailand Research Fund (TRF) for jointly providing her the scholarship that made this study possible.

References

Aviles, J.G.G. and Shepherd, S.A. (1996). Growth and survival of the blue abalone *Haliotis fulgens* in barrel at Cedros Island, Baja, California, with a review of abalone barrel culture. *Aquaculture* 140, 169-176.

Benson, R., Dowsett, M., Grabski, T. and Geedes, P. (2001). The effect of shelter type on growth & survival in juvenile greenlip abalone, *Haliotis laevigata* (Donovan) Curin University of Technology Western Australia. 7 p.

Fallu, R. (1991). Abalone farming. England. Fishing news books. Great Britain.

Handlinger, J., J. Carson, L. Donachie, L. Gabor and D. Taylor. (2002). Bacterial infection in Tasmanian farmed abalone: causes, pathology, farm factors and control options. (Abstract). Handbook and Abstracts, Fifth Symposium on Diseases in Asian Aquaculture, Queensland, Australia, 24-28 November 2002., 139 p.

Hahn, K.O. (1989). *Handbook of Culture of Abalone and Other Marine Gastropods*, CRC Press. Inc. Boca Raton, Florida, USA.

Hindrum, S., Maquire, G., Edwards, S., Burke, C. and John, D. (1998). Is the need for refuges in abalone culture dependent on stocking density for greenlip abalone? Proceedings of the 5th Annual Abalone Aquaculture Workshop. Abalone Aquaculture Sub-Program. Fisheries Research & Development Corporation. July, 1-4.

Huchette, S.M.H., Koh, C.S. and Day, R.W. (2003). The effects of density on the behaviour and growth of juvenile blacklip abalone (*Haliotis rubra*). *Aquaculture International* 11, 411-428.

Jarayabhand, P. and Newkirk, G.F. (1989). Effects of intraspecific competition on growth of the European oyster, *Ostrea edulis* Linnaeus, 1750. *J. Shellfish Res.* 8, 359–365.

Jarayabhand, P. and Paphavasit, N. (1996). A review of the culture of tropical abalone with special reference to Thailand. *Aquaculture* 140, 159-168.

Mgaya, Y.D. and Mercer, J.P. (1995). The effects of size grading and stocking density on the growth of juvenile abalone, *Haliotis tuberculata* Linnaeus. *Aquaculture* 136, 297-312.

Saila, S.B., Recksiek, C.W. and Prager, M.H. (1988). Basic Fishery Science Programs: a Compendium of Micro-computer Programs and Manual of Operation. *Aqua. Fish. Sci.*,18,230 p.

Shepherd, S.A. and Hearn, W.S. (1983) Studies on southern Australian abalone (genus *Haliotis*). IV. Growth of *H. laevigata* and *H. ruber*. *Australian Journal of Marine and Freshwater Research* 34, 461-475.

Taylor J.J., Rose R.A., Southgate P.C. and Taylor C.E. (1997). Effects of stocking density on growth and survival of earl juvenile silver-lip pearl oysters, *Pinctada maxima* (Jameson), held in suspended nursery culture, *Aquaculture* 153, 41-49.

Tookwinas, S., Leknim, W., Donyadol, Y., Preedalampabuttra, Y. and Perngmak P. (1986). Survey on species and distribution of abalone (*Haliotis spp.*) in Surattani, Nakornsrithammarat and Songkhla Provinces. Contribution No. 1/1986, National Institute of Coastal Aquaculture, Department of Fisheries, Thailand, 16 p. (in Thai, with English abstract).

Uki, N. (1989). Abalone seeding production and its theory. 1. *Int. J. Agric. Fish. Technol.*, 1, 3-15.